PATENT
2870-0173P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	HAYASHIZAKI, Yoshihide	Conf.:
Appl. No.:	New	Group:
Filed:	August 24, 2001	Examiner:
For:	METHOD OF PREPARING NORMALIZED AND/OR SUBTRACTED CDNA	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

August 24, 2001

Sir:

The following preliminary amendments and remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE CLAIMS:

Please amend the claims as follows:

4. (Amended) The method of claim 1, wherein in step III), normalization is conducted first, followed by subtraction.

5. (Amended) The method of claim 1, wherein in step III), subtraction is conducted first, followed by normalization.

6. (Amended) The method of claim 1, wherein in step III), said tester and normalization and subtraction drivers are mixed together and normalization and subtraction are conducted as a single step.

7. (Amended) The method of claim 1, wherein said normalized and/or subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

8. (Amended) The method of claim 1, wherein step III) comprises the addition of an enzyme capable of cleaving single-strand RNA driver nonspecifically bound to single strand cDNA and the cleaved single strand RNA driver is removed.

12. (Amended) The method of claim 1, wherein said cDNA tester is prepared by CAP-trapping the 5′ end of RNA.

13. (Amended) The method of claim 1, wherein the preparation of said cDNA tester comprises the following steps:

(1) synthesizing first strand cDNA by means of reverse transcriptase forming mRNA/cDNA hybrids;

(2) chemically binding a tag molecule to the diol structure of the 5′ CAP($^{7Me}G_{ppp}N$) site of mRNA forming hybrids;

(3) trapping long-strand, full-coding, and/or full-length cDNA hybrids; and

(4) removing single strand mRNA through digestion with an enzyme capable of cleaving single strand mRNA.

15. (Amended) The method of claim 1, wherein said polynucleotide driver for normalization and/or subtraction is RNA and/or DNA.

17. (Amended) The method of claim 1, wherein said normalization driver comprises cellular mRNA from the same library, the same tissue, or the same cDNA population as what is to be normalized.

18. (Amended) The method of claim 1, wherein said normalization driver comprises single strand cDNA obtained from the same library, the same tissue, or the same cDNA population as what is to be normalized.

19. (Amended) The method of claim 1, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from what is to be subtracted.

20. (Amended) The method of claim 1, wherein said subtraction driver comprises single strand cDNA from a library, tissue, or cDNA population differing from what is to be normalized.

21. (Amended) The method of claim 1, further comprising a step V) of preparing a second strand of recovered cDNA and performing cloning.

26. (Amended) The method of claim 22, wherein said normalized and/or subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

27. (Amended) The method of claim 22, wherein step III) comprises the addition of an enzyme capable of cleaving single-strand RNA driver nonspecifically bound to single strand cDNA and the cleaved single strand RNA driver is removed.

31. (Amended) The method of claim 22, wherein said cDNA tester is prepared by CAP-trapping the 5' end of RNA.

32. (Amended) The method of claim 22, wherein said normalization driver comprises cellular mRNA from the same library, the same tissue, or the same cDNA population as what is to be normalized.

33. (Amended) The method of claim 22, wherein said normalization driver comprises single strand cDNA obtained from the

same library, the same tissue, or the same cDNA population as what is to be normalized.

34. (Amended) The method of claim 22, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from what is to be subtracted.

35. (Amended) The method of claim 22, wherein said subtraction driver comprises single strand cDNA from a library, tissue, or cDNA population differing from what is to be normalized.

36. (Amended) The method of claim 22, further comprising a step V) of preparing a second strand of recovered cDNA and performing cloning.

41. (Amended) The method of claim 37, wherein said normalized and subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

42. (Amended) The method of claim 37, wherein step III) comprises the addition of an enzyme capable of cleaving single-strand RNA driver nonspecifically bound to single strand cDNA and the cleaved single strand RNA driver is removed.

46. (Amended) The method of claim 37, wherein said cDNA tester is prepared by CAP-trapping 5′ end of RNA.

47. (Amended) The method of claim 37, wherein the preparation of said cDNA tester comprises the following steps:

(1) synthesizing first strand cDNA by means of reverse transcriptase forming mRNA/cDNA hybrids;

(2) chemically binding a tag molecule to the diol structure of the 5′ CAP($^{7Me}G_{ppp}N$) site of mRNA forming hybrids;

(3) trapping long-strand, full-coding, and/or full-length cDNA hybrids; and

(4) removing single strand mRNA through digestion with an enzyme capable of cleaving single strand mRNA.

49. (Amended) The method of claim 37, wherein said polynucleotide driver for normalization and/or subtraction is RNA and/or DNA.

51. (Amended) The method of claim 37, wherein said normalization driver comprises cellular mRNA from the same library, the same tissue, or the same cDNA population as what is to be normalized.

52. (Amended) The method of claim 37, wherein said normalization driver comprises single strand cDNA obtained from the same library, the same tissue, or the same cDNA population as what is to be normalized.

53. (Amended) The method of claim 37, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from what is to be subtracted.

54. (Amended) The method of claim 37, wherein said subtraction driver comprises single strand cDNA from a library, tissue, or cDNA population differing from what is to be normalized.

55. (Amended) The method of claim 37, further comprising a step V) of preparing a second strand of recovered cDNA and performing cloning.

60. (Amended) The method of claim 56, wherein in step c), normalization is conducted first, followed by subtraction.

61. (Amended) The method of claim 56, wherein in step c), subtraction is conducted first, followed by normalization.

62. (Amended) The method of claim 56, wherein in step c), said tester and normalization and subtraction drivers are mixed together and normalization and subtraction are conducted as a single step.

63. (Amended) The method of claim 56, wherein said normalized and/or subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

64. (Amended) The method of claim 56, wherein the enzyme of said step d) is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

65. (Amended) The method of claim 56, wherein the enzyme of said step d) is RNase I.

66. (Amended) The method of claim 56, wherein said cDNA tester is prepared by CAP-trapping the 5′ end of RNA.

67. (Amended) The method of claim 56, further comprising the step g) of preparing a second strand of recovered cDNA and performing cloning.

68. (Amended) The method of claim 1, wherein said tester/driver hybrids are bound to tag molecules.

70. (Amended) The method of claim 1, wherein said tester/driver hybrids are removed through the use of a matrix.

74. (Amended) The method of claim 72, wherein said antibody covering said beads or said antibody binding said beads is an antiantigen antibody, antibiotin antibody, antiavidin antibody, antistreptavidin antibody, or antidigoxigenin antibody.

75. (Amended) The method of claim 1, wherein said tester/driver hybrid is removed through the use of streptavidin/phenol.

76. (Amended) The method of claim 1, wherein hydroxyapadite and nonlabeled RNA are employed to remove said tester/driver hybrid.

80. (Amended) The method of claim 77, wherein said RNA/DNA hybrid is a product of normalization.

81. (Amended) The method of claim 77, wherein said RNA/DNA hybrid is a product of subtraction.

82. (Amended) The method of claim 77, wherein said RNA/DNA hybrid is the product of a method comprising the steps of normalization and subtraction in any order or of a method comprising a single normalization/subtraction step.

85. (Amended) The method of claim 77, wherein said DNA or cDNA is long-chain, full-coding, and/or full-length cDNA.

86. (Amended) The method of claim 1 employed to prepare one, two, or more libraries.

87. (Amended) cDNA or a cDNA library obtainable by the method of claim 1.

Docket No. 2870-0173P

REMARKS

The amendment to the claims is merely to delete multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachment: Version with Markings Showing Changes Made

(Rev. 01/22/01)

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows:

4. (Amended) The method of [any of claims 1-3]claim 1, wherein in step III), normalization is conducted first, followed by subtraction.

5. (Amended) The method of [any of claims 1-3]claim 1, wherein in step III), subtraction is conducted first, followed by normalization.

6. (Amended) The method of [any of claims 1-3]claim 1, wherein in step III), said tester and normalization and subtraction drivers are mixed together and normalization and subtraction are conducted as a single step.

7. (Amended) The method of [any of claims 1-6]claim 1, wherein said normalized and/or subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

8. (Amended) The method of [any of claims 1-7]claim 1, wherein step III) comprises the addition of an enzyme capable of cleaving single-strand RNA driver nonspecifically bound to single strand cDNA and the cleaved single strand RNA driver is removed.

12. (Amended) The method of [any of claims 1-11]claim 1, wherein said cDNA tester is prepared by CAP-trapping the 5′ end of RNA.

13. (Amended) The method of [any of claims 1-12]claim 1, wherein the preparation of said cDNA tester comprises the following steps:

(1) synthesizing first strand cDNA by means of reverse transcriptase forming

mRNA/cDNA hybrids;

(2) chemically binding a tag molecule to the diol structure of the 5′ CAP($^{7Me}G_{ppp}N$)

site of mRNA forming hybrids;.

(3) trapping long-strand, full-coding, and/or full-length cDNA hybrids; and

(4) removing single strand mRNA through digestion with an enzyme capable of

cleaving single strand mRNA.

15. (Amended) The method of [any of claims 1-14]claim 1, wherein said polynucleotide driver for normalization and/or subtraction is RNA and/or DNA.

17. (Amended) The method of [any of claims 1-16]claim 1, wherein said normalization driver comprises cellular mRNA from the same library, the same tissue, or the same cDNA population as what is to be normalized.

18. (Amended) The method of [any of claims 1-16]claim 1, wherein said normalization driver comprises single strand cDNA obtained from the same library, the same tissue, or the same cDNA population as what is to be normalized.

19. (Amended) The method of [any of claims 1-16]claim 1, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from what is to be subtracted.

20. (Amended) The method of [any of claims 1-16]claim 1, wherein said subtraction driver comprises single strand cDNA from a library, tissue, or cDNA population differing from what is to be normalized.

21. (Amended) The method of [any of claims 1-20]claim 1, further comprising a step V) of preparing a second strand of recovered cDNA and performing cloning.

26. (Amended) The method of [any of claims 22-25]claim 22, wherein said normalized and/or subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

27. (Amended) The method of [any of claims 22-26]claim 22, wherein step III) comprises the addition of an enzyme capable of cleaving single-strand RNA driver nonspecifically bound to single strand cDNA and the cleaved single strand RNA driver is removed.

31. (Amended) The method of [any of claims 22-30]claim 22, wherein said cDNA tester is prepared by CAP-trapping the 5′ end of RNA.

32. (Amended) The method of [any of claims 22-31]claim 22, wherein said normalization driver comprises cellular mRNA from the same library, the same tissue, or the same cDNA population as what is to be normalized.

33. (Amended) The method of [any of claims 22-31]claim 22, wherein said normalization driver comprises single strand cDNA obtained from the same library, the same tissue, or the same cDNA population as what is to be normalized.

34. (Amended) The method of [any of claims 22-31]claim 22, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from what is to be subtracted.

35. (Amended) The method of [any of claims 22-31]claim 22, wherein said subtraction driver comprises single strand cDNA from a library, tissue, or cDNA population differing from what is to be normalized.

36. (Amended) The method of [any of claims 22-35]claim 22, further comprising a step V) of preparing a second strand of recovered cDNA and performing cloning.

41. (Amended) The method of [any of claims 37-40]claim 37, wherein said normalized and subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

42. (Amended) The method of [any of claims 37-41]claim 37, wherein step III) comprises the addition of an enzyme capable of cleaving single-strand RNA driver nonspecifically bound to single strand cDNA and the cleaved single strand RNA driver is removed.

46. (Amended) The method of [any of claims 37-45]claim 37, wherein said cDNA tester is prepared by CAP-trapping 5′ end of RNA.

47. (Amended) The method of [any of claims 37-46]claim 37, wherein the preparation of said cDNA tester comprises the following steps:

(1) synthesizing first strand cDNA by means of reverse transcriptase forming

mRNA/cDNA hybrids;

(3) chemically binding a tag molecule to the diol structure of the 5′ CAP($^{7Me}G_{ppp}N$) site of mRNA forming hybrids;

(3) trapping long-strand, full-coding, and/or full-length cDNA hybrids; and

(4) removing single strand mRNA through digestion with an enzyme capable of

cleaving single strand mRNA.

49. (Amended) The method of [any of claims 47-48]claim 37, wherein said polynucleotide driver for normalization and/or subtraction is RNA and/or DNA.

51. (Amended) The method of [any of claims 37-50]claim 37, wherein said normalization driver comprises cellular mRNA from the

same library, the same tissue, or the same cDNA population as what is to be normalized.

52. (Amended) The method of [any of claims 37-50]claim 37, wherein said normalization driver comprises single strand cDNA obtained from the same library, the same tissue, or the same cDNA population as what is to be normalized.

53. (Amended) The method of [any of claims 37-50]claim 37, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from what is to be subtracted.

54. (Amended) The method of [any of claims 37-50]claim 37, wherein said subtraction driver comprises single strand cDNA from a library, tissue, or cDNA population differing from what is to be normalized.

55. (Amended) The method of [any of claims 37-54]claim 37, further comprising a step V) of preparing a second strand of recovered cDNA and performing cloning.

60. (Amended) The method of [any of claims 56-59]claim 56, wherein in step c), normalization is conducted first, followed by subtraction.

61. (Amended) The method of [any of claims 56-59]claim 56, wherein in step c), subtraction is conducted first, followed by normalization.

62. (Amended) The method of [any of claims 56-59]claim 56, wherein in step c), said tester and normalization and subtraction drivers are mixed together and normalization and subtraction are conducted as a single step.

63. (Amended) The method of [any of claims 56-62]claim 56, wherein said normalized and/or subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

64. (Amended) The method of [any of claims 56-65]claim 56, wherein the enzyme of said step d) is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

65. (Amended) The method of [any of claims 56-63]claim 56, wherein the enzyme of said step d) is RNase I.

66. (Amended) The method of [any of claims 56-65]claim 56, wherein said cDNA tester is prepared by CAP-trapping the 5' end of RNA.

67. (Amended) The method of [any of claims 56-66]claim 56, further comprising the step g) of preparing a second strand of recovered cDNA and performing cloning.

68. (Amended) The method of [any of claims 1-67]claim 1, wherein said tester/driver hybrids are bound to tag molecules.

70. (Amended) The method of [any of claims 1-69]claim 1, wherein said tester/driver hybrids are removed through the use of a matrix.

74. (Amended) The method of claim 72 [or 73], wherein said antibody covering said beads or said antibody binding said beads is an antiantigen antibody, antibiotin antibody, antiavidin antibody, antistreptavidin antibody, or antidigoxigenin antibody.

75. (Amended) The method of [any of claims 1-74]claim 1, wherein said tester/driver hybrid is removed through the use of streptavidin/phenol.

76. (Amended) The method of [any of claims 1-75]claim 1, wherein hydroxyapadite and nonlabeled RNA are employed to remove said tester/driver hybrid.

80. (Amended) The method of [any of claims 77-79]claim 77, wherein said RNA/DNA hybrid is a product of normalization.

81. (Amended) The method of [any of claims 77-79]claim 77, wherein said RNA/DNA hybrid is a product of subtraction.

82. (Amended) The method of [any of claims 77-79]claim 77, wherein said RNA/DNA hybrid is the product of a method comprising the steps of normalization and subtraction in any order or of a method comprising a single normalization/subtraction step.

85. (Amended) The method of [any of claims 77-84]claim 77, wherein said DNA or cDNA is long-chain, full-coding, and/or full-length cDNA.

86. (Amended) The method of [any of claims 1-85]claim 1 employed to prepare one, two, or more libraries.

87. (Amended) cDNA or a cDNA library obtainable by [any of] the [methods] method of [claims 1-86] claim 1.